PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :		(11) International Publication Number:	WO 00/37103
A61K 39/00	A2	(43) International Publication Date:	29 June 2000 (29.06.00)

(21) International Application Number: PCT/US99/28211

(22) International Filing Date: 29 November 1999 (29.11.99)

(30) Priority Data:
09/217,037 21 December 1998 (21.12.98) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 09/217,037 (CON)
Filed on 21 December 1998 (21.12.98)

(71) Applicant *(for all designated States except US)*: XAVOS [US/US]; 2995 Woodside Road #400, Woodside, CA 94062–2446 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: WEBB, Robert, R. [US/US]; 802 Stetson Street, Moss Beach, CA 94038 (US). MCKEE, Constance, A. [US/US]; 2995 Woodside Road #400, Woodside, CA 94062–2446 (US).

(74) Agent: WEITZ, David, J.; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304–1050 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: COMPOUNDS FOR INTRACELLULAR DELIVERY OF THERAPEUTIC MOIETIES TO NERVE CELLS

(57) Abstract

A compound for delivering a non–cytotoxic therapeutic moiety into nerve cells, the compound having the general formula : B–L–TM where: B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell; TM is a therapeutic moiety which has a non–cytotoxic therapeutic effect when absorbed by a nerve cell; and L is a linker coupling B to TM.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

Compounds For Intracellular Delivery Of Therapeutic Moieties To Nerve Cells

Field of the Invention

The present invention relates to compounds which can be used to selectively deliver moieties to nerve cells. More specifically, the invention relates to compounds which include a therapeutic moiety and facilitate absorption of the therapeutic moiety by nerve cells.

Background of the Invention

Our understanding of the structure and function of the nervous system has been greatly advanced owing to enormous progresses made in field of neuroscience. Cellular and molecular mechanisms of neuron growth and development and diseases associated with the central and peripheral nervous systems are studied extensively by using rapidly growing techniques in molecular and cell biology. However, a need still exists for efficacious treatments of many neurological disorders including Alzheimer's disease, Parkinson's disease, Huntington's disease, schizophrenia, severe pain, multiple sclerosis, bipolar disease, and diseases of the nervous system due to infection by viruses and other microorganisms (herpes simplex, HIV, cytomegalovirus, parasites, fungi, prion, etc.).

Many neuropharmaceutical agents have been developed to treat diseases of the nervous system, but their usefulness has been hampered by severe side effects partially due to nonspecific interactions between these agents and cells or tissues other than the targeted cells. For example, steroid hormone cortisone and its derivatives are widely used to treat inflammation in the body including the nerve system to reduce symptoms such as swelling, tenderness and pain. However, the steroid dosage has to be kept at the lowest effective level because of its severe side effects. Steroid hormone binds to its cognate nuclear hormone

receptor and induces a cascade of cellular effects, including programmed cell death of the neurons in the brain (Kawata M., et al., J. Steroid Biochem. Mol. Biol. 65: 273-280 (1998)). Since steroid hormone receptors, such as glucocorticord receptor for cortisone, distribute in a wide variety of tissues and cells, nonspecific interactions of the hormone with its cognate receptor in different sites is unavoidable if the drug is circulated systemically.

A need continues to exist for an effective system for delivering therapeutic agents selectively to nerve cells and nerve tissues. Various techniques have been developed to deliver drugs, but with only limited success. For example, liposomes have been used as carrier molecules to deliver a broad spectrum of agents including small molecules, DNAs, RNAs, and proteins. Liposome mediated delivery of pharmaceutical agents has major drawbacks because of its lack of target specificity. Attempts have been made to overcome this problem by covalently attaching whole site-specific antibody or Fab fragments to liposomes containing a pharmaceutical agent (Martin et al., Biochem. 20, 4229-4238, (1981)). However, an intrinsic problem of particular importance in any liposome carrier system is that in most cases the targeted liposome does not selectively reach its target site *in vivo*. Whether or not liposomes are coated with antibody molecules, liposomes are readily phagocytosed by macrophages and removed from circulation before reaching their target sites.

Summary of the Invention

Compounds of the present invention include compounds having the general formula:

B-L-M

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

M is a moiety which performs a useful non-cytotoxic function when absorbed by a nerve cell; and

L is a linker coupling **B** to **M**.

In one embodiment, the compounds have the general formula:

B-L-TM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

TM is a therapeutic moiety which has a non-cytotoxic therapeutic effect when absorbed by a nerve cell; and

L is a linker coupling **B** to **TM**.

In another embodiment, the compounds have the general formula:

B-L-IM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

IM is a non-cytotoxic imaging moiety which can be used to image a nerve cell or an intracellular component of the nerve cell; and

L is a linker coupling **B** to **IM**.

In regard to each of the above embodiments, particular classes of binding agents **B** which may be used include, but are not limited to, nucleic acid sequences, peptides, peptidomimetics, antibodies and antibody fragments. Examples of nucleic acids that can serve as the binding agent **B** include, but are not limited to, DNA and RNA ligands that function as antagonists of nerve growth factors or inhibit binding of other growth factors to nerve cell surface receptors. Examples of peptides that can serve as the binding agent **B** include, but are not limited to, members of the nerve growth factors (neurotrophin) family such as NGF, BDNF, NT-3, NT-4, NT-6; derivatives, analogs, and fragments of nerve growth factors such as recombinant molecules of NGF and BDNF; and synthetic peptides that bind to nerve cell surface receptors and have agonist or antagonist activities of nerve growth factors.

Antibodies, derivatives of antibodies and antibody fragments can also serve as the binding agent **B**. Examples of this type of binding agent **B** include, but are not limited to, anti-human trkA monoclonal antibody 5C3 and anti-human p75 monoclonal antibody MC192.

The therapeutic moiety **TM** is selected to perform a non-cytotoxic therapeutic function within nerve cells. Examples of non-cytotoxic functions which the therapeutic moiety **TM** may perform include, but are not limited to, the functions performed by adrenergic agents, analgesics, anti-trauma agents, anti-viral agents, gene therapy agents, and hormones (growth factors, interferons, etc.). Examples of classes of therapeutic moieties include, but are not limited to, adrenergic agents (e.g., epinephrine, norepinephrine, dopamine, etenolol), analgesics (e.g.,

opioids, codeine, oxycodone), anti-trauma agents, anti-viral agents (e.g., acyclovir, gancyclovir, AZT, ddI, ddC, etc.), gene therapy agents (e.g., DNAs or RNAs which introduce a gene or replace a mutated gene), steroids (e.g., cortisone, progesterone, estrogen), and hormones (e.g., growth factors, interferons).

The imaging moiety **IM** is a non-cytotoxic agent which can be used to locate and optionally visualize a nerve cell or an internal component of the nerve cell which has absorbed the imaging moiety. Fluorescent dyes may be used as an imaging moiety **IM**. Radioactive agents which are non-cytotoxic may also be an imaging moiety **IM**.

In general, the linker may be any moiety which can be used to link the binding agent **B** to the moiety **M**. In one particular embodiment, the linker is a cleavable linker. The use of a cleavable linker enables the moiety **M** linked to the binding agent **B** to be released from the compound once absorbed by the nerve cell. The cleavable linker may be cleaved by a chemical agent, enzymatically, due to a pH change, or by being exposed to energy. Examples of forms of energy which may be used include light, microwave, ultrasound, and radiofrequency.

The present invention also relates to a method for selectively delivering a moiety into nerve cells comprising the steps of:

delivering to a patient a compound having the general formula:

B-L-M

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

M is a moiety which performs a useful non-cytotoxic function when absorbed by a nerve cell; and

L is a linker coupling **B** to **M**.

having the compound selectively bind to a nerve cell surface receptor via the binding agent **B**; and

having the compound be absorbed by the nerve cell mediated by the binding of the binding agent **B** to the nerve cell surface receptor.

In one embodiment, moiety **M** is a therapeutic moiety **TM** as described herein and in another embodiment is an imaging moiety **IM**.

The above method can be used to deliver therapeutic moieties for treating a variety of neurological disorders when the therapeutic moiety **TM** is a moiety useful for treating such neurological disorders.

The above method can be used to deliver therapeutic moieties for treating pain when a therapeutic moiety **TM** for treating pain, such as an analgesic, is included as the therapeutic moiety **TM** in the compound.

The above method can also be used to deliver steroid hormones for treating nerve damage when a therapeutic moiety **TM** for treating nerve damage, such as a steroid hormone, is included as the therapeutic moiety **TM** in the compound.

The above method can also be used to stimulate nerve growth when a therapeutic moiety **TM** for inducing the production of a nerve growth factor is included as the therapeutic moiety **TM** in the compound.

The above method can also be used to treat infected nerve cells infected with viruses or immunize nerve cells from viruses when the therapeutic moiety **TM** in the compound is an antiviral agent.

The above method can also be used to perform gene thereapy when the therapeutic moiety **TM** is a gene therapy agent.

Detailed Description

The present invention relates to compounds which include a binding agent which binds to a nerve cell surface receptor and facilitates absorption of the compound by the nerve cell; and a moiety. Different Moieties may be included in the compounds of the present invention including therapeutic moieties that are non-cytotoxic to the nerve cells and imaging moieties which can be used to image nerve cells which absorb these compounds.

In one embodiment, compounds of the present invention have the general formula:

B-L-TM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

TM is a therapeutic moiety which has a non-cytotoxic therapeutic effect when absorbed by a nerve cell; and

L is a linker coupling **B** to **TM**.

According to this embodiment, the binding agent **B** serves as a homing agent for nerve cells by selectively binding to nerve cell surface receptors. The binding agent **B** also serves to facilitate absorption of the compound by the nerve cell. The binding agent **B** can be any molecule which can perform these two functions. Particular classes of binding agents which may be used include, but are not limited to, nucleic acid sequences, peptides, peptidomimetics, antibodies and antibody fragments.

Examples of nucleic acids that can serve as the binding agent **B** include, but are not limited to, DNA and RNA ligands that function as antagonists of nerve growth factors or inhibit binding of other growth

factors to nerve cell surface receptors (Binkley, J., et al., Nucleic Acid Res. 23: 3198-3205 (1995); Jellinek, D., et al., Biochem. 33:10450-10456 (1994)).

Examples of peptides that can serve as the binding agent **B** include, but are not limited to, members of the nerve growth factors (neurotrophin) family such as NGF, BDNF, NT-3, NT-4, NT-6, etc. (see reviews: Frade, J. M., et al., Bioessays 20: 137-145 (1998); Shieh, P. B., Curr. Biol. 7: R627-R630 (1997); Dechant, G., et al., Curr. Opin. Neurobiol. 7: 413-418 (1997); Chao, M. V. and Hempstead, B. L., Trends Neurobiol. 18: 321-326 (1995)); and derivatives, analogs, and fragments of nerve growth factors such as recombinant molecules of NGF and BDNF (Ibanez et al., EMBO J. 10: 2105-2110; Ibanez et al., EMBO J. 12: 2281-2293), synthetic peptides that bind to nerve cell surface receptors and have agonist or antagonist activities of nerve growth factors (Longo, F. M., et al., Cell Regulation 1: 189-195 (1990); LeSauteur, L. et al., J. Biol. Chem. 270: 6564-6569 (1995); Longo F. M., et al., J. Neurosci. Res. 48: 1-17; Longo, et al., Nature Biotech. 14: 1120-1122 (1997)).

Examples of antibodies, derivatives of antibodies and antibody fragments that can serve as the binding agent **B** include, but are not limited to, anti-human trkA monoclonal antibody 5C3 (Kramer, K., et al., Eur. J. Cancer 33: 2020-2091 (1997)), anti-human p75 monoclonal antibody MC192 (Maliatchouk, S. and Saragovi, H. U., J. Neurosci. 17: 6031-7).

According to this embodiment, the therapeutic moiety **TM** is selected to perform a non-cytotoxic therapeutic function within nerve cells. Examples of non-cytotoxic functions which the therapeutic moiety **TM** may perform include, but are not limited to, the functions performed by analgesics, anti-trauma agents, anti-viral agents, gene therapy agents, and hormones (growth factors, interferons, etc.). Examples of classes of therapeutic moieties include, but are not limited to, adrenergic agents (e.g., epinephrine, norepinephrine, dopamine, etenolol), analgesics (e.g., opioids, codeine, oxycodone), anti-trauma agents, anti-viral agents (e.g., acyclovir, gancyclovir, AZT, ddI, ddC, etc.), gene therapy agents (e.g.,

DNAs or RNAs which introduce a gene or replace a mutated gene), steroids (e.g., cortisone, progesterone, estrogen), and hormones (e.g., growth factors, interferons).

The linker **L** serves to link the binding agent **B** to the therapeutic moiety **TM**. A wide variety of linkers are known in the art for linking two molecules together, particularly, for linking a moiety to a peptide or nucleic acid, all of which are included within the scope of the present invention.

Examples of classes of linkers that may be used to link the binding agent **B** to the therapeutic moiety **TM** include amide, alkylamine, thiolether, alkyl, cycloalkyl, aryl linkages such as those described in Hermanson, G.T., Bioconjugate Techniques (1996), Academic Press, San Diego, CA.

In certain applications, it is desirable to release the therapeutic moiety **TM** once the compound has entered the nerve cell, resulting in a release of the therapeutic moiety **TM**. Accordingly, in one variation, the linker **L** is a cleavable linker. This enables the therapeutic moiety **TM** to be released from the compound once absorbed by the nerve cell. This may be desirable when the therapeutic moiety **TM** has a greater therapeutic effect when separated from the binding agent. The therapeutic moiety **TM** may have a better ability to be absorbed by an intracellular component of the nerve cell when separated from the binding agent. Accordingly, it may be necessary or desirable to separate the therapeutic moiety **TM** from the compound so that the therapeutic moiety **TM** can enter the intracellular compartment.

Cleavage of the linker releasing the therapeutic moiety may be as a result of a change in conditions within the nerve cells as compared to outside the nerve cells, for example, due to a change in pH within the nerve cell. Cleavage of the linker may occur due to the presence of an enzyme within the nerve cell which cleaves the linker once the compound enters the nerve cell. Alternatively, cleavage of the linker may occur in response to energy or a chemical being applied to the nerve cell. Examples of types of energies that may be used to effect cleavage of the

linker include, but are not limited to light, ultrasound, microwave and radiofrequency energy.

The linker **L** used to link the binding agent **B** to the therapeutic moiety **TM** may be a photolabile linker. Examples of photolabile linkers include those linkers described in US Patent No. 5,767,288 and No. 4,469,774. The linker **L** used to link the binding agent **B** to the therapeutic moiety **TM** may also be an acid labile linker. Examples of acid labile linkers include linkers formed by using cis-aconitic acid, cis-carboxylic alkatriene, polymaleic anhydride, and other acidlabile linkers, such as those linkers described in US Patent Nos. 5,563,250 and 5,505, 931.

Further examples of cleavable linkers include, but are not limited to the linkers described in Lin, et al., J. Org. Chem. 56:6850-6856 (1991); Ph.D. Thesis of W.-C. Lin, U.C. Riverside, (1990); Hobart, et al., J. Immunological Methods 153: 93-98 (1992) ; Jayabaskaran, et al., Preparative Biochemistry 17(2): 121-141 (1987); Mouton, et al., Archives of Biochemistry and Biophysics 218: 101-108 (1982) ; Funkakoshi, et al., J. of Chromatography 638:21-27 (1993); Gildea, et al., Tetrahedron Letters 31: 7095-7098 (1990); WO 85/04674; and Dynabeads™ (Dynal, Inc., 5 Delaware Drive, Lake Success, NY 11042).

In another embodiment, compounds of the present invention have the general formula:

B-L-IM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

IM is a non-cytotoxic imaging moiety which can be used to image the nerve cell or an intracellular component of the nerve cell; and

L is a linker coupling **B** to **IM**.

According to this embodiment, the binding agent **B** and linker **L** may be varied as described above with regard to compounds having the general formula **B-L-TM**. Further according to this embodiment, the imaging moiety **IM** may be a non-cytotoxic moiety which can be used to image nerve cells. Examples of imaging moieties that may be used include fluorescent dyes and radioisotopes which are non-cytotoxic.

The present invention also relates to a method for selectively delivering a non-cytotoxic therapeutic moiety into nerve cells comprising the steps of:

delivering to a patient a therapeutic amount of a compound having the general formula:

B-L-TM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell,

TM is a therapeutic moiety which has a non-cytotoxic therapeutic effect when absorbed by a nerve cell, and

L is a linker coupling **B** to **TM**;

having the compound selectively bind to a nerve cell surface receptor via the binding agent **B**; and

having the compound be absorbed by the nerve cell mediated by the binding of the binding agent **B** to the nerve cell surface receptor.

The method of the present invention offers the advantage of specifically targeting a non-cytotoxic therapeutic moiety to nerve cells where the therapeutic moiety is absorbed by the nerve cells. The method utilizes the fact that internalization of the conjugated agent is mediated by the binding of the binding agent **B** to nerve cell surface receptors. Once internalized, the therapeutic moiety can accumulate within the nerve cells where it has a therapeutic effect. The ability to selectively deliver the

compound to nerve cells reduces the overall amount of therapeutic moiety which needs to be administered. Selective delivery of the therapeutic moiety to the nerve cell reduces the amount of side effects observed due to non-specific administration of the therapeutic moiety. In addition, the therapeutic moiety is less likely to be separated from the binding agent and non-specifically administered as compared to delivery methods involving the use of a binding agent and a therapeutic moiety in combination.

The method of the present invention can be used to deliver therapeutic moieties for treating a variety of neurological disorders including, but not limited to, Alzheimer's disease, Parkinson's disease, multiple sclerosis, neurodegenerative disease, epilepsy, seizure, migraine, trauma and pain. Examples of neuropharmaceuticals that may be used include proteins, antibiotics, adrenergic agents, anticonvulsants, nucleotide analogs, anti-trauma agents, peptides and other classes of agents used to treat or prevent a neurological disorders. For example, analgesics such as opioids, codeine and oxycodone can be conjugated to the binding agent **B** and specifically delivered to the nerve cells. Since the same level of pain relief can be achieved using a smaller dosage of analgesics, side effects such as respiratory depression or potential drug addiction can be avoided or at least ameliorated. Steroid hormones such as corticosteriods can also be conjugated with nerve cell-specific binding agents and used to treat inflammation of the nerves, which may reduce the side effects associated with high doses of steroids, such as weight gain, redistribution of fat, increase in susceptibility to infection, and avascular necrosis of bone.

The method according to the present invention can also be used to deliver agents that induce the production of nerve growth factor in the target nerve cells, especially under conditions of pathogenic under-expression of NGFs (See Riaz, S. S. and Tomlinson, D. R. Prog. Neurobiol. 49: 125-143 (1996)). NGF induction has been demonstrated in a wide variety of cell types, such as fibroblasts (Furukawa, Y. et al., FEBS Lett. 247: 463-467(1989)), astrocytes (Furukawa, Y. et al., FEBS Lett. 208: 258-262 (1986)), Schwann cells (Ohi, T. et al., Biochem. Int. 20:739-

746 (1990)) with a variety of agents including cytokines, steriods, vitamins, hormones, and unidentified components of serum. Specific examples of agents known to induce NGF include 4-methylcatechol, clenbuterol, isoprenaline, L-tryptophan, 1,25-dihydroxyvitamin D3, forskolin, fellutamide A, gangliosides and quinone derivatives (Riaz, S. S. and Tomlinson, D. R. Prog. Neurobiol. 49: 125-143 (1996)).

The method according to the present invention can also be used to deliver antiviral drugs into nerve cells in order to treat diseases caused by viral infection, to eliminate viruses spread to the nerves, and to inhibit infection by such viruses. Examples of viruses that infect the nervous system include but are not limited to rabies viruses, herpes viruses, polioviruses, arboviruses, reoviruses, pseudorabies, corona viruses, and Borna disease viruses. For example, antiviral drugs such as acyclovir, gancyclovir, and Cifodovir can be conjugated to the binding agent and used to inhibit active or latent herpes simplex viruses in the peripheral and central nervous system. Specific delivery of the conjugate containing these antiviral drugs to the nervous system can reduce the side effects associated with high doses or long-term administration of these drugs, such as headaches, rash and paresthesia.

The method according to the present invention can also be used to deliver marker compounds to image intracellular components of the nerve cells. Such marker compounds include but are not limited to fluorescent dyes, radioactive complexes, and other luminophores.

The method according to the present invention can also be used to perform gene therapy wherein nucleic acids (DNA or RNA) are delivered to the nerve cells. These nucleic acids may serve to replace genes which are either defective, absent or otherwise not properly expressed by the patient's nerve cell genome.

The above and other features and advantages of the present invention will become more apparent in the following description of the preferred embodiments in greater detail.

1. Binding Agent (B)

According to the present invention, a compound with a binding agent **B** is used to selectively deliver the conjugated therapeutic moieties **TM** to nerve cells. At the nerve cell, the binding agent **B** interacts with a receptor on the nerve cell and is absorbed by the nerve cell mediated by this interaction. Any molecules possessing these two physical properties are intended to fall within the scope of a binding agent **B** as it is used in the present invention. In particular, peptides or proteins with these features can serve as a binding agent **B**, examples including but not limited to nerve growth factors (neurotrophins), antibodies against nerve cell-specific surface proteins, mutants and synthetic peptides derived from these peptides or proteins.

In one embodiment, neurotrophins are preferably used as the binding agent **B**. Neurotrophins are a family of small, basic polypeptides that are required for the growth, development and survival of neurons. A particular "survival" factor is taken up by the neuron via binding to one or more of a related family of transmembrane receptors. Table **I** lists several members of the neurotrophin family and their cognate receptors.

As listed in Table **1**, nerve growth factor (NGF) is the first identified and probably the best characterized member of the neurotrophin family. It has prominent effects on developing sensory and sympathetic neurons of the peripheral nervous system. Brain-derived neurotrophic factor (BDNF) has neurotrophic activities similar to NGF, and is expressed mainly in the CNS and has been detected in the heart, lung, skeletal muscle and sciatic nerve in the periphery (Leibrock, J. et al., Nature, 341:149-152 (1989)). Neurotrophin-3 (NT-3) is the third member of the NGF family and is expressed prodominantly in a subset of pyramidal and granular neurons of the hippocampus, and has been detected in the cerebellum, cerebral cortex and peripheral tissues such as liver and skeletal muscles (Ernfors, P. et al., Neuron 1: 983-996 (1990)). Neurotrophin-4 (also called NT-4/5) is

the most variable member of the neurotrophin family. Neurotrophin-6 (NT-6) was found in teleost fish and binds to p75 receptor.

As listed in Table **1** at least two classes of transmembrane glycoproteins (trk and p75) have been identified which serve as receptors for neurotrophins. The trk receptors (tyrosine kinase-containing receptor) bind to neurotrophins with high affinity, whereas the p75 receptors possess lower affinity to neurotrophins. For example, nerve growth factor (NGF) binds to a relatively small number of trkA receptors with high affinity (K_D = 10^{-11}) and to more abundant p75 with lower affinity (K_D = 10^{-9}). The receptor-bound NGF is internalized with membrane-bound vesicles and retrogradely transported the neuronal cell body. Thus, native neurotropins may serve as the binding agent **B** in the compound according the present invention to deliver the conjugated therapeutic agent **TM** to the neuronal cell body.

Table **1** The Neurotrophin Family and Its Receptors.

	Receptor		
Factor	Kinase isoforms	Nonkinase forms	Responsive neurons (examples)
NGF	trkA	p75	Cholinergic forebrain neurons
			Sympathetic ganglia
			DRG nociceptive
BDNF	trkB	$p75^{LNTR}$	Many CNS populations
		$trkB_{T1}$	Vestibular ganglia
		$trkB_{T2}$	Nodose ganglia
			DRG mechanoreceptors
NT-3	trkC	$p75^{LNTR}$	Many CNS populations
	trkB and trkA		Choclear ganglia
	Nonpreferred	$trkC_{TK-113}$	DRG proprioceptive
		$trkC_{TK-108}$	
NT-4	trk B	p75	Many CNS populations
		$trkB_{T1}$	Nodose ganglia
		$trkB_{T2}$	Petrosalganglia
NT-6	trkA	p75	

In addition to the neurotrophins described above, analogs and derivatives of neurotrophins may also serve as the binding agent **B**. The structure of mouse NGF has been solved by X-ray crystallography at 2.3 A

resolution (McDonald et al., Nature, 345: 411-414, (1991)). Murine NGF is a dimeric molecule, with 118 amino acids per protomer. The structure of the protomer consists of three antiparallel pairs of beta strands that form a flat surface, four loop regions containing many of the variable residues between different NGF-related molecules, which may determine the different receptor specificities, and a cluster of positively charged side chains, which may provide a complementary interaction with the acidic low-affinity NGF receptor. Murine NGF has a tertiary structure based on a cluster of three cysteine disulfides and two extended, but distorted beta-hairpins. One of these β-hairpin loops was formed by the NGF 29-35 region. Structure/function relationship studies of NGF and NGF-related recombinant molecules demonstrated that mutations in NGF region 25-36, along with other β-hairpin loop and non-loop regions, significantly influenced NGF/NGF-receptor interactions (Ibanez et al., EMBO J., 10, 2105-2110, (1991)). Small peptides derived from this region have been demonstrated to mimic NGF in binding to trkA receptor and affecting biological responses (LeSauteur et al. J. Biol. Chem. 270, 6564-6569, 1995). Dimers of cyclized peptides corresponding to β-loop regions of NGF were found to act as partial NGF agonists in that they had both survival-promoting and NGF-inhibiting activity while monomer and linear peptides were inactive (Longo et al., J. Neurosci. Res., 48, 1-17, 1997). Cyclic peptides have also been designed and synthesized to mimic the β-loop regions of NGF, BDNF, NT3 and NT-4/5. Certain monomers, dimers or polymers of these cyclic peptides may have a three-dimensional structure which binds to neurotrophin receptors under physiological conditions. All of these structural analogs of neurotrophins that bind to nerve cell surface receptors and are internalized can serve as the binding agent **B** of the compound according to the present invention to deliver the conjugated therapeutic moiety **TM** to the nervous system.

Alternatively, antibodies against nerve cell surface receptors that are capable of binding to the receptors and being internalized can also serve as the binding agent **B**. For example, monoclonal antibody (MAb) 5C3 is

specific for the NGF docking site of the human p140 trkA receptor, with no cross-reactivity with human trkB receptor. MAb 5C3 and its Fab mimic the effects of NGF in vitro, and image human trk-A positive tumors *in vivo* (Kramer et al., Eur. J. Cancer, 33, 2090-2091, (1997)). Molecular cloning , recombination, mutagenesis and modeling studies of Mab 5C3 variable region indicated that three or less of its complementarity determining regions (CDRs) are relevant for binding to trkA. Assays with recombinant CDRs and CDR-like synthetic polypeptides demonstrated that they had agonistic bioactivities similar to intact Mab 5C3. Monoclonal antibody MC192 against p75 receptor has also been demonstrated to have neurotrophic effects. Therefore, these antibodies and their functionally equivalent fragments can also serve as the binding agent **B** of the compound according to the present invention to deliver the conjugated therapeutic agent **TM** into the nerve cells.

Alternatively, peptidomimetics that are synthesized by incorporating unnatural amino acids or other organic molecules may serve as the binding agent **B** of the compound according to the present invention to deliver the conjugated therapeutic agent **TM** into the nerve cells. These synthetic peptide mimics are capable of binding to the nerve cell surface receptor and being internalized into the cell.

It is noted that the identification and selection of moieties which can serve as binding agents in the present invention can be readily performed by attaching an imaging moiety **IM** to the potential binding agent in order to detect whether the potential binding agent is internalized by the nerve cells. In this regard, combinatorial and mutagenesis approaches may be used to identify analogs, derivatives and fragments of known binding moieties which may also be used as binding moieties according to the present invention.

2. Therapeutic Moiety (TM)

An aspect of the present invention relates to the delivery of compounds into nerve cells which are non-cytotoxic to the nerve cells and perform a therapeutic function. Examples of therapeutic functions include, but are not limited to, treatment of neurological disorders, gene therapy, intracellular target imaging, cell sorting, or separation schemes. Examples of classes of therapeutic moieties include, but are not limited to adrenergic agents such as epinephrine, norepinephrine, dopamine, etenolol; analgesics such as opioids, codeine, oxycodone; anti-trauma agents; anti-viral agents such as acyclovir, gancyclovir, AZT, ddI, ddC; gene therapy agents such as; steroids such as cortisone, progesterone, estrogen; and hormones such as growth factors and interferons. Such compounds may optionally also include an imaging moiety, such as fluorescent moieties, for imaging intracellular components of the nerve cells.

3. Linker (L)

According to the present invention, a binding agent **B** is linked to a therapeutic moiety **TM** by a linker **L**. In general, any method of linking a binding agent to a therapeutic moiety may be used and is intended to fall within the scope of the present invention.

Many different types of linkers have been developed for cross linking proteins and conjugating proteins or peptides with other agents. These linkers include zero-length cross linkers, homobifunctional cross-linkers, heterobifunctional cross-linkers and trifunctional cross-linkers. These linkers may have different susceptibility to cleavage under certain conditions. Depending on a particular application according to the present invention, an appropriate linker may be chosen. When an intracellular release of the agent from its conjugate is desired, a cleavable linker is chosen which is susceptible to cleavage by external stimuli such as light

and heat, by intracellular enzymes, or by a particular microenvironment inside the cell.

In one embodiment, the linker **L** has one of the following general structures:

B-R_1-(CO)-NH –R_2-**TM**

B-R_3-NH-R_4-**TM**

B-R_3-S –R_4-**TM**

B-R_5-$(CH_2)_n$ -R_6-**TM**

Wherein R_1, R_2, R_3, R_4, R_5, and R_6 are independently selected from the group consisting of alkyls, aryls, heteroaryls, cycloalkyls, cycloalkenes and heterocycloalkenes.

4. Cleavable Linkers

One particular embodiment of the present invention relates to compounds which include a cleavable linker **L**. In some instances, the therapeutic moiety **TM** is more efficacious or potent when free from a carrier molecule such as a binding agent. In such instances, it is desirable to utilize a cleavable linker which allows the therapeutic moiety **TM** to be released from the compound once inside the cell.

Many cleavable linker groups have been developed which are susceptible to cleavage and by a wide variety of mechanisms. For example, linkers have been developed which may be cleaved by reduction of a disulfide bond, by irradiation of a photolabile bond, by hydrolysis of derivatized amino acid side chain, by serum complement-mediated hydrolysis, and by acid-catalyzed hydrolysis.

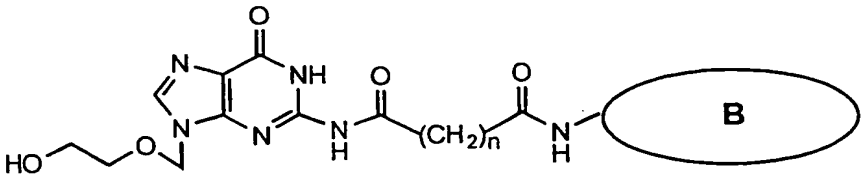
Examples of photolabile linkers that may be used include those linkers described in U.S. Patent Nos. 5,767,288 and No. 4,469,774.

Acid-labile linkers are preferred in the practice of the present invention by taking advantage of a cell's receptor-mediated endocytosis pathways. Receptors that are internalized by receptor-mediated endocytosis pass through acidified compartments known as endosomes or receptosomes. Since the interior of the endosomal compartment is kept acidic (pH~6.0) by ATP-driven H^+ pumps in the endosomal membrane that pump H^+ into the lumen from the cytosol, a change in pH within the nerve cell can be used to cause the acid-labile linker to be cleaved and release the therapeutic moiety. Examples of acid labile linkers which may be used include the cis-aconitic acid, cis-carboxylic alkatriene, polymaleic anhydride, and other acid labile linkers described in US Patent Nos. 5,563,250 and 5,505, 931.

5. Examples Of Compounds According To The Present Invention

Table **2** provides several compounds according to the present invention. It is noted that in each instance, the particular therapeutic moieties, binding moieties, and linkers shown may be interchanged with other suitable therapeutic moieties, binding moieties, and linkers. In this regard, the compounds shown in the table are intended to illustrate the diversity of compounds provided according to the present invention.

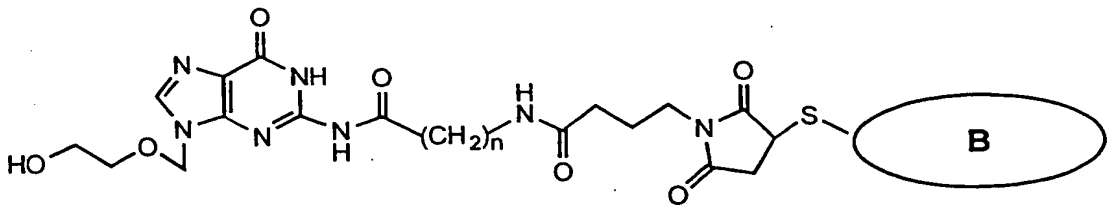
TABLE 2



Acyclovir

wherein

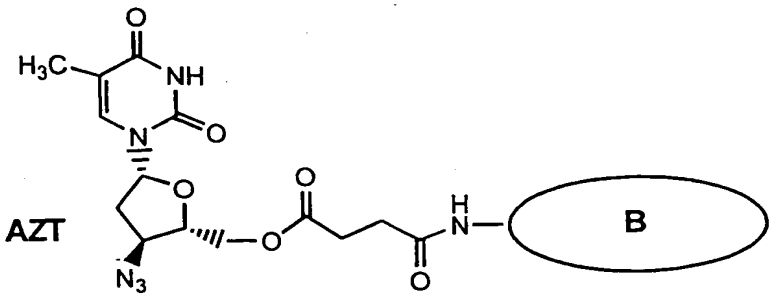
B is selected from the group consisting of nerve growth factors NGF, BDNF, NT-3, NT-4, NT-6, anti-neurotrophin receptor antibodies MAb 5C3 and Mab MC192.



Acyclovir

wherein

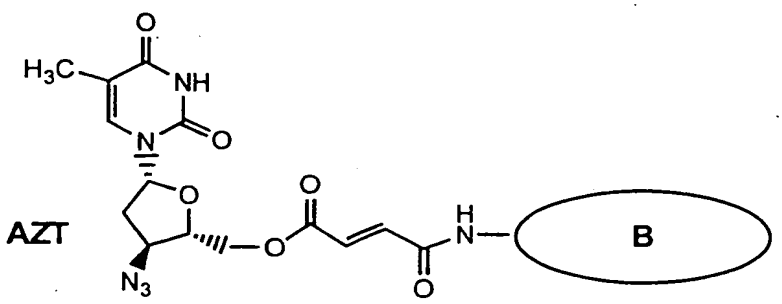
B is selected from the group consisting of nerve growth factors NGF, BDNF, NT-3, NT-4, NT-6, anti-neurotrophin receptor antibodies MAb 5C3 and Mab MC192.



wherein

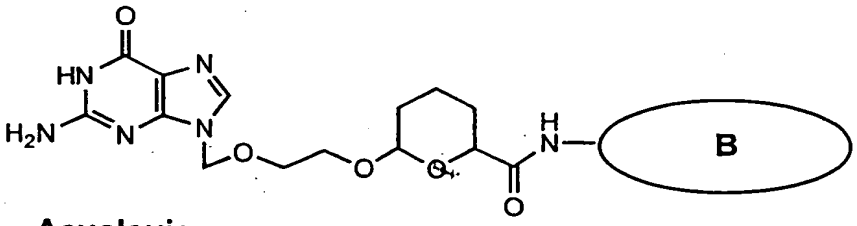
B is selected from the group consisting of nerve growth factors NGF, BDNF, NT-3, NT-4, NT-6, anti-neurotrophin receptor antibodies MAb 5C3 and Mab MC192.

TABLE 2-continued



wherein

B is selected from the group consisting of nerve growth factors NGF, BDNF, NT-3, NT-4, NT-6, anti-neurotrophin receptor antibodies MAb 5C3 and Mab MC192.



Acyclovir

wherein

B is selected from the group consisting of nerve growth factors NGF, BDNF, NT-3, NT-4, NT-6, anti-neurotrophin receptor antibodies MAb 5C3 and Mab MC192.

6. Methods For Using Compounds Of The Pr sent Invention

Described below are several methods for formulating and administering the compounds of the present invention. The compounds of the present invention may be employed in these and other applications.

a. Pharmaceutical Formulations Utilizing Compositions Of The Present Invention

The compounds of the present invention may be incorporated into a variety of pharmaceutical compositions including, but not limited to: a sterile injectable solution or suspension; hard or soft gelatin capsules; tablets; emulsions; aqueous suspensions, dispersions, and solutions; suppositories. Other pharmaceutically suitable formulations for delivering the compounds of the present invention to nerve cells may also be used and are intended to fall within the scope of the present invention.

b. Routes of Administration

The compounds according to the present invention can be administered orally, by subcutaneous or other injection, intravenously, intracerebrally, intramuscularly, parenternally, transdermally, nasally or rectally. The form in which the compound is administered depends at least in part on the route by which the compound is administered.

While the present invention is disclosed with reference to preferred embodiments and examples detailed above, it is to be understood that these examples are intended in an illustrative rather than limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, which modifications will be within the spirit of the invention and the scope of the appended claims. The patents, papers, and books cited in this application are to be incorporated herein in their entirety.

CLAIMS

We claim:

1. A compound for delivering a non-cytotoxic therapeutic moiety into nerve cells, the compound having the general formula:

B-L-TM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

TM is a therapeutic moiety which has a non-cytotoxic therapeutic effect when absorbed by a nerve cell; and

L is a linker coupling **B** to **TM**.

2. The compound according to claim 1 wherein the binding agent **B** is selected from the group consisting of a nucleic acid sequence, a peptide, a peptidomimetic, an antibody and an antibody fragment.

3. The compound according to claim 1 wherein the binding agent **B** is selected from the group consisting of nerve growth factors and analogs, derivatives and fragments of nerve growth factors

4. The compound according to claim 1 wherein the binding agent **B** is selected from the group consisting of antibodies and antibody fragments that selectively bind to nerve cell surface receptors.

5. The compound according to claim 1 wherein the binding agent **B** is a DNA or RNA ligand that functions as an antagonist of nerve growth

factors or inhibits binding of other growth factors to nerve cell surface receptors.

6. The compound according to claim 1 wherein the binding agent **B** is a synthetic peptide that binds to nerve cell surface receptors and has agonist or antagonist activity of nerve growth factors.

7. The compound according to claim 1 wherein the binding agent **B** is selected from the group consisting of anti-human trkA monoclonal antibody 5C3 and anti-human p75 monoclonal antibody MC192.

8. The compound according to claim 1 wherein the binding agent **B** is selected from the group consisting of NGF, BDNF, NT-3, NT-4, NT-6.

9. The compound according to claim 1 wherein the therapeutic moiety **TM** performs a non-cytotoxic function within the nerve cell selected from the group consisting of the functions performed by analgesics, adrenergic agents, anti-trauma agents, anti-viral agents, gene therapy agents, and hormones.

10. The compound according to claim 1 wherein the therapeutic moiety **TM** is selected from the group consisting of analgesics, adrenergic agents, anti-trauma agents, anti-viral agents, gene therapy agents, and hormones.

11. The compound according to claim 1 wherein the linker **L** is a cleavable linker.

12. The compound according to claim 11 wherein the cleavable linker **L** is cleaved when exposed to a particular chemical agent.

13. The compound according to claim 11 wherein the cleavable linker **L** is cleaved when exposed to an enzyme within the nerve cell.

14. The compound according to claim 11 wherein the cleavable linker **L** is a cleavable by a change in pH due to entry of the compound into the nerve cell.

15. The compound according to claim 11 wherein the cleavable linker **L** is cleaved when the nerve cell is exposed to a form of energy.

16. The compound according to claim 15 wherein the form of energy is selected from the group consisting of light, microwave, ultrasound, and radiofrequency.

17. A method for selectively delivering a therapeutic moiety into nerve cells comprising the steps of:

delivering to a patient a compound having the general formula:

B-L-TM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

TM is a therapeutic moiety which has a non-cytotoxic therapeutic effect when absorbed by a nerve cell; and

L is a linker coupling **B** to **M**.

having the compound selectively bind to a nerve cell surface receptor via the binding agent **B**; and

having the compound be internalized by the nerve cell.

18. The method according to claim 17 wherein the method is for treating a neurological disorder, the therapeutic moiety **TM** being selected to be a moiety useful for treating a neurological disorder.

19. The method according to claim 17 wherein the method is for treating pain, the therapeutic moiety **TM** being selected to be a moiety useful as an analgesic.

20. The method according to claim 17 wherein the method is for stimulating nerve growth, the therapeutic moiety **TM** being selected to be a moiety useful for inducing the production of a nerve growth factor is included as the therapeutic moiety **TM** in the compound.

21. The method according to claim 17 wherein the method is for treating infected nerve cells, the therapeutic moiety **TM** being selected to be an antiviral agent.

22. The method according to claim 17 wherein internalization of the compound by the nerve cell is mediated by the binding agent B to the nerve cell surface receptor.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 47/48, A61P 25/00, 25/04 — A3

(11) International Publication Number: **WO 00/37103**

(43) International Publication Date: 29 June 2000 (29.06.00)

(21) International Application Number: PCT/US99/28211

(22) International Filing Date: 29 November 1999 (29.11.99)

(30) Priority Data:
09/217,037 21 December 1998 (21.12.98) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 09/217,037 (CON)
Filed on 21 December 1998 (21.12.98)

(71) Applicant *(for all designated States except US)*: XAVOS [US/US]; 2995 Woodside Road #400, Woodside, CA 94062–2446 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: WEBB, Robert, R. [US/US]; 802 Stetson Street, Moss Beach, CA 94038 (US). MCKEE, Constance, A. [US/US]; 2995 Woodside Road #400, Woodside, CA 94062–2446 (US).

(74) Agent: WEITZ, David, J.; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304–1050 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(88) Date of publication of the international search report:
19 October 2000 (19.10.00)

(54) Title: COMPOUNDS FOR INTRACELLULAR DELIVERY OF THERAPEUTIC MOIETIES TO NERVE CELLS

(57) Abstract

A compound for delivering a non–cytotoxic therapeutic moiety into nerve cells, the compound having the general formula : B–L–TM where: B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell; TM is a therapeutic moiety which has a non–cytotoxic therapeutic effect when absorbed by a nerve cell; and L is a linker coupling B to TM.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

Inte. .tional Application No
PCT/US 99/28211

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/48 A61P25/00 A61P25/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, BIOSIS, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 32738 A (DOLLY JAMES OLIVER ;WHEELER LARRY ALLEN (US); ALLERGAN INC (US); A) 7 December 1995 (1995-12-07) abstract page 1, line 5 - line 7 page 2, line 17 -page 3, line 6 page 11 -page 13; table 1	1,2,9-22
Y	page 14, line 17 -page 16, line 16; claims 1,2	4,5,7
X	WO 97 23608 A (VIAGENE INC) 3 July 1997 (1997-07-03) page 2, line 24 -page 4, line 31	1-3,6, 8-17,22
Y	page 17, line 13 - line 19; claims 1-3	4,5,7

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 June 2000	20/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Niemann, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/US 99/28211

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 07092 A (UNIV MEDICINE AND DENTISTRY OF) 16 March 1995 (1995-03-16) page 7, line 1 - line 11 page 9, line 16 - line 29 page 23, line 1 -page 25, line 17	1-3,6, 8-10, 17-20,22
Y	page 30, line 22 - line 27; claims 1-3,8-11	4,5,7

Y	KRAMER K ET AL: "Monoclonal antibody to human Trk-A: Diagnostic and therapeutic potential in neuroblastoma." MEETING ON ADVANCES IN NEUROBLASTOMA RESEARCH;PHILADELPHIA, PENNSYLVANIA, USA; MAY 22-25, 1996, vol. 33, no. 12, October 1997 (1997-10), pages 2090-2091, XP002139706 European Journal of Cancer Oct., 1997 ISSN: 0959-8049 cited in the application abstract	4,7

Y	MALIARTCHOUK SERGEI ET AL: "Optimal nerve growth factor trophic signals mediated by synergy of TrkA and p75 receptor-specific ligands." JOURNAL OF NEUROSCIENCE, vol. 17, no. 16, 1997, pages 6031-6037, XP002140658 ISSN: 0270-6474 cited in the application the whole document	4,7

Y	BINKLEY JONATHAN ET AL: "RNA ligands to human nerve growth factor." NUCLEIC ACIDS RESEARCH 1995, vol. 23, no. 16, 1995, pages 3198-3205, XP002139707 ISSN: 0305-1048 cited in the application abstract	5

A	US 5 833 988 A (FRIDEN PHILLIP M) 10 November 1998 (1998-11-10) abstract column 1, line 65 -column 2, line 27 column 2, line 60 -column 4, line 12 column 6, line 33 - line 52; claims 1-6	1-22

A	US 5 502 037 A (KONDRATYEV ALEXI) 26 March 1996 (1996-03-26) abstract column 1, line 1 -column 5, line 47; claim 1	1-22

	-/--	

4

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte. ..ional Application No
PCT/US 99/28211

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropnate, of the relevant passages	Relevant to claim No.
A	US 5 767 288 A (STOWELL MICHAEL H B ET AL) 16 June 1998 (1998-06-16) cited in the application the whole document	11-16
A	US 5 563 250 A (FITZNER JEFFREY N ET AL) 8 October 1996 (1996-10-08) cited in the application the whole document	11-16
A	US 5 505 931 A (PRIBISH JAMES R) 9 April 1996 (1996-04-09) cited in the application the whole document	11-16

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. ..tional Application No
PCT/US 99/28211

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9532738 A	07-12-1995	AU 695623 B	20-08-1998
		AU 2622295 A	21-12-1995
		CA 2191754 A	07-12-1995
		DE 69511860 D	07-10-1999
		DE 69511860 T	10-02-2000
		EP 0760681 A	12-03-1997
		ES 2138740 T	16-01-2000
		JP 10500988 T	27-01-1998
WO 9723608 A	03-07-1997	NONE	
WO 9507092 A	16-03-1995	AU 7564194 A	27-03-1995
US 5833988 A	10-11-1998	US 5527527 A	18-06-1996
		US 5182107 A	26-01-1993
		US 5154924 A	13-10-1992
		US 5672683 A	30-09-1997
		US 5977307 A	02-11-1999
		AT 131070 T	15-12-1995
		AU 654115 B	27-10-1994
		AU 6445990 A	08-04-1991
		CA 2066244 A	08-03-1991
		DE 69024057 D	18-01-1996
		DE 69024057 T	13-06-1996
		EP 0490998 A	24-06-1992
		ES 2080838 T	16-02-1996
		JP 5500944 T	25-02-1993
		WO 9103259 A	21-03-1991
US 5502037 A	26-03-1996	AU 7320394 A	06-02-1995
		CA 2166798 A	19-01-1995
		EP 0712313 A	22-05-1996
		JP 9503486 T	08-04-1997
		WO 9501806 A	19-01-1995
US 5767288 A	16-06-1998	NONE	
US 5563250 A	08-10-1996	US 5141648 A	25-08-1992
		US 5017693 A	21-05-1991
		WO 9014844 A	13-12-1990
		DE 3873887 A	24-09-1992
		DE 3873887 T	04-02-1993
		EP 0318948 A	07-06-1989
		JP 1294638 A	28-11-1989
US 5505931 A	09-04-1996	AU 4660093 A	24-01-1994
		AU 6020494 A	26-09-1994
		CA 2139323 A	06-01-1994
		CA 2157402 A	15-09-1994
		CN 1092076 A	14-09-1994
		EP 0653941 A	24-05-1995
		EP 0687261 A	20-12-1995
		FI 946156 A	27-02-1995
		HU 71220 A	28-11-1995
		JP 7508979 T	05-10-1995
		JP 8507517 T	13-08-1996
		MX 9303963 A	31-01-1995
		NO 945093 A	27-02-1995

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. ..ional Application No
PCT/US 99/28211

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5505931 A		WO 9400145 A	06-01-1994
		WO 9420487 A	15-09-1994